



SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND LINEZOLID IN PURE AND COMBINE DOSAGE FORM

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ABSTRACT

Two simple, rapid, accurate and precise spectrophotometric methods have been developed for simultaneous estimation of Cefixime Trihydrate (CEF) and Linezolid (LINE) in their combine dosage form. Method I, absorbance ratio method, involves formation of absorbance ratio equation at 281.6 nm (isoabsorptive point) and 289.4 nm (λ_{max} of CEF). Method II, combination of second derivative dual wavelength and zero crossing point method, involves difference in absorbance at 283.8 nm and 301.8 nm (difference is zero for CEF) used for estimation of LINE and measurement of absorbance at 301 nm (zero crossing point of LINE) for estimation of CEF in second derivative spectra. Developed methods were validated according to ICH guidelines. The calibration graph follows Beer's law in the range of 6-18 $\mu\text{g/ml}$ for LINE and 2-6 $\mu\text{g/ml}$ for CEF with R^2 value greater than 0.999. Accuracy of all methods was determined by recovery studies and showed % recovery between 98 to 102%. Intraday and inter day precision was checked for all methods and mean %RSD was found to be less than 2 for all methods. The methods were successfully applied for estimation of LINE and CEF in marketed formulation.

Keywords: Linezolid, Cefixime Trihydrate, Absorbance ratio method, Second derivative, zero crossing point method.

INTRODUCTION

Cefixime (as Trihydrate or anhydrous) is an oral third generation cephalosporin antibiotic. Chemically, it is (6*R*,7*R*)-7-[[2-(2-amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Figure 1), clinically used in the treatment of susceptible infection including gonorrhea, otitis media, pharyngitis, lower respiratory tract infection such as bronchitis and urinary tract infection. It acts by inhibiting cell wall synthesis. It binds to one of the penicillin binding proteins (PBPs) which inhibit the final transpeptidation step of the peptidoglycan synthesis in the bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death. Linezolid, (*S*)-*N*-[3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl)acetamide (Figure 2), is a synthetic antibiotic belonging to a new class of antimicrobial called the oxazolidinones. It is active against most gram positive bacteria including streptococci, methicillin resistant staphylococcus aureus and vancomycin resistant enterococci. It acts by inhibiting initiation of bacterial protein synthesis.¹⁻⁴

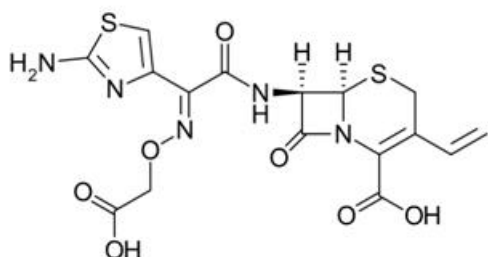


Figure 1: CEFIXIME

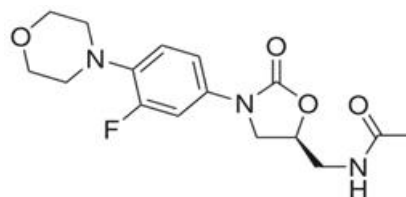


Figure 2: LINEZOLID

Objective of study

A survey of analytical literature for CEFIXIME revealed that methods based on Spectrophotometric and RP-HPLC method for simultaneous determination of Cefixime with Ofloxacin, Potassium clavulanate and with Moxifloxacin. Spectrofluorimetric method of Cefixime through derivatization with 2-cyanoacetamide and stability indicating analytical method has been available in literature. RP-HPLC for determination of Linezolid in plasma, Chiral-HPLC and stability indicating LC for enantiomeric separation of Linezolid and its impurity have been reported. The objective of present study was to develop rapid, accurate, economic, precise and specific analytical methods for simultaneous estimation of Cefixime and Linezolid in pure and combine dosage form.⁵⁻¹²

MATERIALS AND METHODS

Apparatus and Software

Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells

over the range of 200-400 nm. The samples were weighed on electronic analytical balance (A×120, Shimadzu).

Reagent and Chemicals

Methanol for UV spectroscopy (Spectrochem Pvt. Ltd, Mumbai, India) was used as solvent.

Preparation of solution

Accurately weighed Linezolid and Cefixime Trihydrate equivalent to anhydrous Cefixime (in quantity of 25mg and 28mg respectively) were transferred to two separate 25ml volumetric flask, dissolved with the use of methanol and volume was made up to mark with methanol to obtain stock solution of LINE(1000 µg/ml) and CEF(1000 µg/ml). From these stock solutions, LINE (100 µg/ml) and CEF(50 µg/ml) were prepared by transferring 5ml and 2.5ml aliquots respectively to other 50ml volumetric flask and make up the volume with methanol. From this, 6-18 µg/ml of LINE and 2-6 µg/ml of CEF were prepared in 10ml volumetric flask using methanol.

Method I- Absorbance Ratio Method

The absorbance ratio method is a modification of the simultaneous equation method. It depends on the property that, for a substance which obeys Beer's Law at all wavelengths, the ratio of absorbance at any two wavelengths is a constant value independent of concentration or pathlength. This method uses the ratio of absorbance at two selected wavelengths, one at isoabsorptive point and other is being λ_{max} of one of two compounds. From stock solution, standard solution of LINE (6-18 µg/ml) and CEF (2-6 µg/ml) were prepared by appropriate dilutions and were scanned in the entire UV range 200-400 nm and isoabsorptive point and λ_{max} were determined (Figure-3). LINE and CEF have λ_{max} at 257.4 nm and 289.4 nm, respectively. Both the drugs were found to have same absorbance at 281.6 nm (isoabsorptive point). The wavelengths selected for analysis were 281.6 nm (isoabsorptive point) and 289.4 nm (λ_{max} of CEF). Calibration graph of absorbance versus concentration at 281.6 nm (isoabsorptive point) and at 289.4 nm (λ_{max} of CEF) are shown in Figure 4.1 and 4.2, respectively.¹³

The concentration of two drugs in mixture was calculated by using following equations:

$$C_{(LINE)} = [(QM - QY) / (QX - QY)] \times A1/aX1$$

$$C_{(CEF)} = [(QM - QX) / (QY - QX)] \times A1/aY1$$

Where; QM = A2/A1, QX = aX2/aX1, QY = aY2/aY1; 1 designates isoabsorptive point and 2 designates λ_{max} of CEF; aX1 and aX2 is absorptivity of LINE at 1 and 2 wavelength respectively; aY1 and aY2 is absorptivity of CEF at 1 and 2 wavelength respectively; A1 and A2 are absorbance of the mixture at 1 and 2 wavelength respectively.

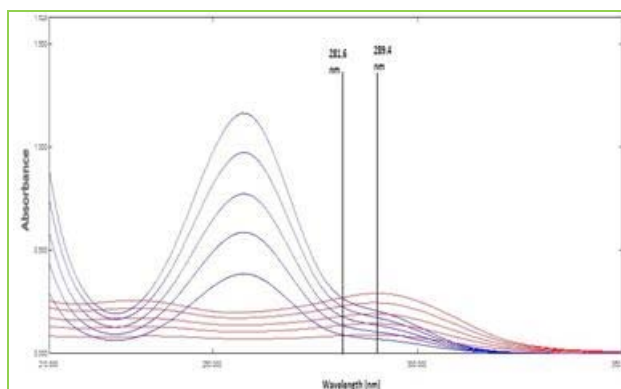


Figure 3: Overlain spectra of LINE (6-18 µg/ml, blue) and CEF (2-6 µg/ml, red)

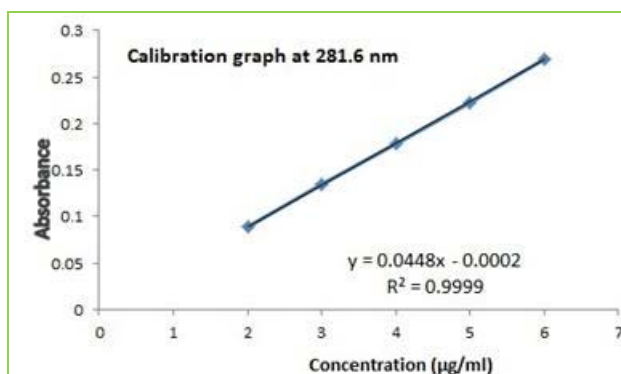


Figure 4.1: Calibration graph at 281.6 nm (isoabsorptive point) for absorbance ratio method

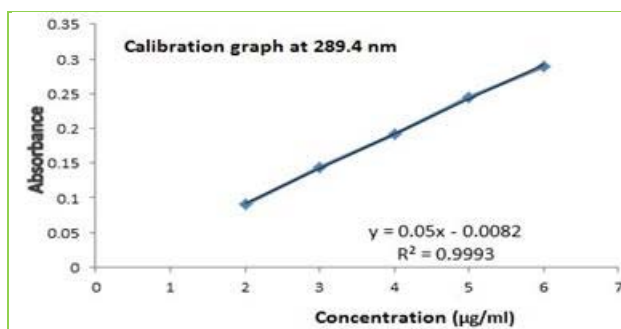


Figure 4.2: Calibration graph at 289.4 nm (λ_{max} of CEF) for absorbance ratio method

Method II - Second Derivative Dual wavelength and Zero Crossing point Method

The absorption spectra of LINE (6-18 µg/ml) and CEF (2-6 µg/ml) were recorded in the entire UV range 200-400 nm and were stored in the memory of the instrument and transformed to second derivative spectrum with $\Delta\lambda=16$ nm and scaling factor 100 (Figure 5). At 283.8 nm and 301.8 nm difference in absorbance is zero for CEF so this can be used for estimation of LINE. Difference in absorbance at 283.8 nm and 301.8 nm of LINE were plotted against concentration for preparation of calibration graph (Figure 6.1).

At 301 nm LINE is having zero crossing point and CEF can be estimated at this point. The absorbance at 301 nm was plotted against the respective concentration of CEF for preparation of calibration graph (Figure 6.2).

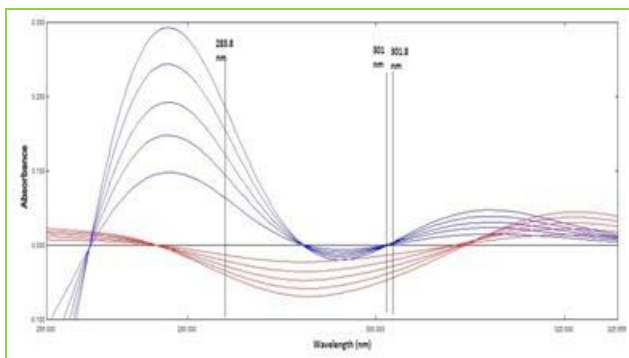


Figure 5: Second derivative overlay spectra of LINE (6-18 µg/ml, blue) and CEF (2-6 µg/ml, red)

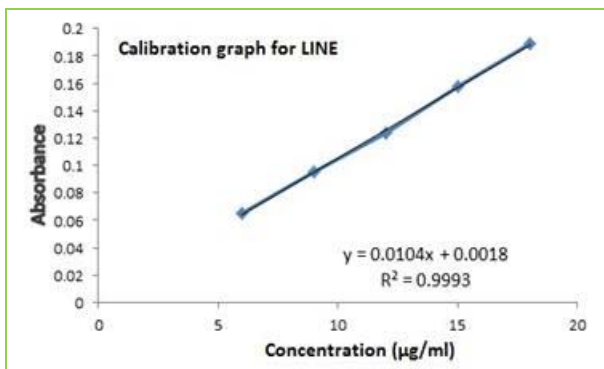


Figure 6.1: Calibration graph of LINE for second derivative dual wavelength method

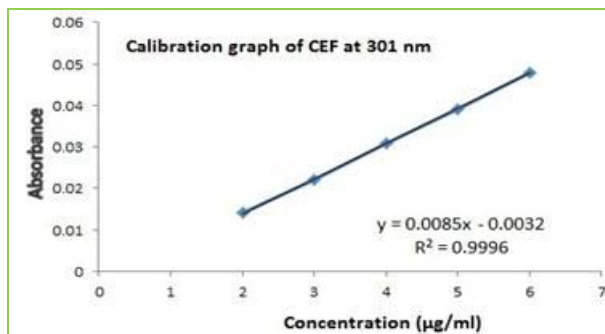


Figure 6.2: Calibration graph of CEF at 301 nm for second derivative dual wavelength method

Assay of marketed formulation by Method I and II

20 tablets were powdered and an amount equivalent to 200 mg CEF and 600 mg LINE was weighed and dissolved in 25 ml methanol. Solutions were filtered using Whatman filter paper grade 1. Appropriate dilutions were prepared in methanol taking suitable aliquots of the clear filtrates and subjected to analysis using all the three methods described above. The result of analysis is reported in table 1.

Table 1: Result of simultaneous estimation of marketed formulation for method I and II

Formulation : ZIFITURBO			
Drugs	Label claim	% Drug found ± SD*	
		Method I	Method II
LINE	600 mg	100.14±0.0761	99.80±0.0316
CEF	200 mg	99.97±0.0397	100.83±0.0134

*Mean value of five determinations.

Table 2: Result of recovery study of LINE and CEF by developed method

Method	% Spiking	C _{Added}		C _{Found}		% Recovery ± SD	
		LINE	CEF	LINE	CEF	LINE	CEF
A	50	3	1	9.0633	3.0008	100.70±.0648	100.02±.0414
	100	6	2	12.0568	4.0193	100.47±.0296	100.48±.0238
	150	9	3	15.2056	5.0059	101.37±.0694	100.11±.0674
B	50	3	1	9.0638	3.0431	100.70±.0870	101.43±.0678
	100	6	2	11.9807	4.0317	99.83±.0333	100.79±.0142
	150	9	3	15.0384	5.0166	100.25±.0333	100.39±.0591

Table 3: Summary of validation parameter by developed methods

Parameter	Method I		Method II	
	LINE	CEF	LINE	CEF
Analytical wavelength	281.6 nm and 289.4 nm		283.8 and 301.8 nm	301 nm
Beer's range (µg/ml)	6-18	2-6	6-18	2-6
Correlation coefficient	0.9999	0.9993	0.9993	0.9996
Intraday precision* (%RSD)	0.6264	0.7654	0.5826	0.7259
Inter day precision* (%RSD)	0.7252	0.8753	1.0286	1.1759
LOD (µg/ml)	0.2435	0.2877	0.1142	0.3466
LOQ (µg/ml)	0.7380	0.8719	0.3486	1.0505

*Mean value of three determinations.

RESULTS AND DISCUSSION

The proposed methods of analysis for LINE and CEF in combination were validated as per the recommendations of ICH guidelines for parameter like accuracy, precision, linearity, range, limit of detection and limit of quantification. The drugs obeys Beer's law in concentration range of 6-18 µg/ml for LINE and 2-6 µg/ml for CEF with correlation coefficient greater than 0.999. The results of marketed formulation analysis are shown in table 1. Results of recovery studies were within 98-102%, shown in table 2. Precision of both the methods were calculated by intraday and inter day variation study and %RSD of observation were found to be less than 2, shown in table 3.¹⁴

CONCLUSION

Two spectrophotometric methods were developed for simultaneous estimation of LINE and CEF in their combined formulation without prior separation. Methods were found to be simple, rapid, economic, accurate and precise. The results of validation tests were found to be satisfactory and therefore, these methods can be applied successfully for routine quality control analysis of LINE and CEF in bulk and pharmaceutical formulation.

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